

Formamido Remnant of Thymine, Sep. 13, 1992, "Free Rad. Res. Comms.", vol. 18, No. 1, pp. 17-28.

Although the above embodiments and examples are described relative various reagents and enzymes, the invention is not limited to such. For example, Protamine Sulphate or other binding or immobilization techniques or solutions known in the art may be substituted for Reacti-bind. Other ELISA-like or colorimetric analysis techniques may be employed. Tagging or labeling may be accomplished with residues other than biotin. Other control DNA may be used beside calf thymus against which to compare the sample DNA. Washing may be performed using solutions other than buffered Tween 20 detergent. Comparing may be accomplished by other techniques, e.g., absorbance, optical density, color, etc., the object being to provide a basis for comparing sample and control DNA. Ordering of the steps described may be altered. Based on the foregoing description, one skilled in the art can easily ascertain modifications, substitutes, applications, and/or variations. It is the intent to include such modifications, substitutes, applications, and/or variations without departing from the spirit and scope of the invention.

What is claimed is:

1. A method of assaying DNA comprising:

(i) binding to an analysis plate both sample DNA under examination and control DNA having known abasic sites,

(ii) reacting the abasic sites with an aldehyde group-specific chemical reagent selected from a group of reagents including an aldehyde reactive probe (ARP) (N'-aminooxymethylcarbonylhydrazino-D-biotin) reagent whereby to attach the ARP to abasic sites,

(iii) using an ELISA-like method to detect abasic sites tagged with biotin after the reacting step wherein the ELISA-like method includes an avidin-biotin-complex conjugated with horseradish peroxidase or alkali phosphatase.

2. The method as recited in claim 1, comprising reacting ARP with AP sites of DNA of cells in culture before the binding step.

wherein the sample and control DNA are tagged or labeled separately with a the residue of the ARP reagent and then bound to the analysis plate for comparison.

3. The method as recited in claim 1, wherein the control DNA is a depurination of calf thymus.

4. The method as recited in claim 1, wherein the steps performed relative to the sample DNA and the control DNA are performed simultaneously so as to remove environmental or process variables at the comparing step.

5. The method as recited in claim 1, wherein Reacti-bind is used during the binding step.

6. The method as recited in claim 1, further including a washing step after the binding and labeling steps.

7. A method of quantitatively assaying DNA damage comprising the steps of binding to an analysis plate sample DNA and multiple control DNA specimens each having of known number of abasic sites, tagging aldehyde groups associated with abasic sites of the sample and control DNA, performing an ELISA-like method to obtain one of absorbance, optical density, and color density of the sample DNA and control DNA specimens, and comparing the sample DNA with multiple control DNA specimens to determine the number of abasic sites in the sample DNA.

8. A method of assaying repair capacity of sample DNA comprising:  
treating sample and control DNA specimen with an enzyme that produces a substrate to which ARP attaches, reacting the sample and specimen DNA with ARP,

tagging aldehyde groups associated with abasic sites of the sample and control DNA,

performing an ELISA-like method to obtain at least one of absorbance, optical density, and color density of the sample DNA and control DNA specimens, and

comparing at least one of color, optical density, and absorbance of sample DNA with multiple control DNA specimens to determine relative enzyme activity levels of the sample and control DNA.

9. The method as recited in claim 8 wherein the treating step includes using an enzyme selected from the group including Endonuclease III, 8-oxoguanine glycosylase [yOGG1], human 8-oxoguanine glycosylase [hOGG1].

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10. A method of assaying DNA comprising:

(i) binding sample and control DNA to an analysis plate to a microtiter plate using Reacti-bind wherein the control DNA has a known number of abasic sites,

10 (ii) removing the excess Reacti-bind and unbound DNA using a Tween 20 buffered detergent so as not to remove the bound DNA,

(iii) reacting the bound DNA with an excess amount of aldehyde reactive probe (ARP) reagent,

(iv) removing the excess and unreacted ARP from the analysis plate,

15 (v) labeling/tagging the attached ARP using a biotinylated chemical agent, and

(vi) performing a colorimetric analysis to quantitatively assess the sample DNA relative to the control DNA attached to the plate.

20 11. The method as recited in claim 10, further including binding to the analysis plate a relatively high percentage of DNA contained in a solution of relatively low concentration being in the range of 1.0 to 10.0 nanograms of DNA per millilitre.

25 12. A simplified kit for assaying sample DNA comprising:  
an analysis plate, such as a microtiter plate;  
a number of control DNA specimens of known concentration of abasic sites;

a surface treatment solution to enhance attachment of the DNA to the  
30 analysis plate;

a washing detergent for removing excess material from the plate;

an aldehyde reactive probe (ARP) to label or tag abasic sites while attached to the plate;

a colorimetric test kit such as a kit for performing an avidin-biotin horseradish peroxidase technique; and

5 instructions to provide a colorimetric comparison between sample DNA and control DNA by binding to an analysis plate sample DNA and multiple control DNA specimens each having of known number of abasic sites, tagging aldehyde groups associated with abasic sites of the sample and control DNA, performing an ELISA-like method to obtain at least one of absorbance, optical  
10 density, and color density of the sample DNA and control DNA specimens, and comparing the sample DNA with multiple control DNA specimens to determine the number of abasic sites in the sample DNA.

13. The simplified kit as recited in claim 12 further comprising a Reacti-bind  
15 solution for binding DNA to the analysis plate and an avidin biotin horseradish peroxidase enzyme for comparing enzyme digestion activity relative to the sample and control DNA.

14. A method for determining DNA repair capacity comprising the steps of:  
20 subjecting sample and control DNA to a substrate specific repair enzyme, tagging the product of the enzyme reaction, binding the DNA to an analysis plate, and determining the resulting number of abasic sites remaining on the analysis plate after the enzyme reaction whereby to assay the ability of the cell to undergo  
25 DNA repair.

15. The method as recited in claim 14, further comprising the steps of  
subjecting the sample and control DNA to a DNA glycosylase selected from the  
group of endonuclease III, N-glycosylase, 8-oxoguanine, alkA protein, and other  
30 broad and narrow spectrum DNA glycosylase.

16. An apparatus that automates assaying of DNA comprising:  
an analysis plate to which sample and control DNA is bound,  
a number of processing stations that perform process activities with  
respect to the analysis plate including at least one of binding DNA to a plate,  
5 reacting DNA with an aldehyde reactive probe reagent, tagging the ARP reagent,  
washing the DNA with a detergent, incubating the sample and control DNA, and  
a controller that effect movement of the plate to the processing stations to  
perform the steps of binding to the analysis plate sample DNA and multiple  
control DNA specimens of known number of abasic sites, tagging aldehyde  
10 groups associated with abasic sites of the sample and control DNA, performing an  
ELISA-like method to obtain at least one of absorbance, optical density, and color  
of the sample DNA and control DNA specimens, and outputting an indication of a  
comparison of color density of the sample DNA relative to the control DNA  
specimens to indicate the number of abasic sites in the sample DNA.

17. The apparatus as recited in claim 16, wherein the controller effects  
operations of the processing stations to dispense sample and control DNA on the  
analysis plate; react a surface treatment agent including Reacti-bind with the  
sample and control DNA to bind them to the plate; wash the plate so as to remove  
20 excess surface treatment agent; reacting the bound DNA with an aldehyde  
reactive probe (ARP) so as to tag or label the DNA bound to the plate;  
biotinilating the ARP-tagged DNA; washing excess un-reacted ARP from the  
plate without removal of bound DNA; and outputting an indication of a  
colorimetric technique including an avidin-biotin horseradash peroxidase  
25 technique to indicate the number/concentration of abasic sites in the sample DNA.